Dr. Sharon M. Douglas
Department of Plant Pathology and Ecology
The Connecticut Agricultural Experiment Station
123 Huntington Street, P. O. Box 1106
New Haven, CT 06504

Phone: (203) 974-8601
Fax: (203) 974-8502
Email: Sharon.Douglas@po.state.ct.us

RAMORUM BLIGHT (a.k.a. SUDDEN OAK DEATH) IN CONNECTICUT

Sudden Oak Death is the popular name for an emergent and potentially destructive new disease in the United States. The name, Sudden Oak Death (SOD), was initially used to describe the rapid decline of many native tree species first observed by hikers in coastal California forests in the mid 1990s. Trees in decline included tanoak (*Lithocarpus densiflorus*), California coast live oak (*Quercus agrifolia*), and California black oak (*Quercus kelloggii).* In some sites, 100% of mature tanoaks and 45% of California coast live oaks had been killed by this mysterious disease. An unidentified fungus-like organism was isolated from infected trees by forest pathologists in 2000 but the identity of this organism (*Phytophthora ramorum)* was not confirmed until 2001. To date, natural infections are limited to the West Coast, which includes several coastal counties in California and one county in Oregon. More recently, *P. ramorum* has also been detected in nurseries in California, Washington, Oregon, and British Columbia. National concern regarding this disease was heightened during 2004 when several shipments of plants from nurseries in California and Oregon were distributed to many states, including Connecticut, and were later determined to test positive for *P. ramorum.* As a result of these contaminated shipments, there is justifiable concern that Connecticut forests and landscapes may have been exposed to this pathogen.

The significant devastation of tanoaks and other species in California forests, the discovery of a closely related strain of the pathogen in Europe, and our lack of experience in dealing with this relatively new organism foster high levels of anxiety and uncertainty in scientific and public communities nationally and internationally. We are unable to make accurate predictions with regard to spread and potential environmental impact of this introduced pathogen because of this limited understanding. Many have asked if *P. ramorum* could be as destructive as the organisms responsible for Chestnut Blight, White Pine Blister Rust, or Dutch Elm Disease. Although we know that these three non-native, introduced pathogens have a history of destroying chestnuts, white pines, and elms in forests and landscapes, it is just too early to assess the potential of *P. ramorum*. Quite simply, we don't know enough about the biology and genetics of this plant pathogen to answer this and many other questions. We need to proceed with caution.

CAUSAL AGENT

Phytophthora ramorum is a fungus-like organism, or oomycete, identified as the causal agent of SOD in 2001. A very similar organism had been reported as the cause of a foliar blight of rhododendron and viburnum in Germany and Holland in 1993 but had not been characterized at the time of the report. However, in 2001, scientists determined the two pathogens were the same species and named the organism. To date, two races or mating types of the pathogen have been described. Race A1 is commonly found in Europe and Race A2 is predominant in the United States, although isolated reports of A1 have been reported in several nurseries in the United States. *P. ramorum* is not a pathogen of humans or animals. In Connecticut, many other species of *Phytophthora* are commonly found in landscapes, forests, and production nurseries. Most of these are root-infecting, soil-borne, and water-borne organisms with somewhat limited capability for aerial spread. However, while maintaining the soil-borne and water-borne characteristics of its relatives, *P. ramorum* also appears to have a high capacity for significant aerial dispersal. It is this potential for aerial spread that makes this pathogen particularly destructive. *P. ramorum* produces several different morphological structures that are important for spread and survival, including sporangia, zoospores, and chlamydospores. Abundant sporangia (microscopic, football-shaped structures, often arranged in grape-like clusters on stalks) are produced aerially on the surfaces of infected plants. These structures are easily dislodged by wind, water, and physical contact. Sporangia produce the microscopic, biflagellate swimming spores called zoospores that are capable of moving to other plants through water, soil, and splashing rain and irrigation water. Zoospores are fragile and short-lived. Chlamydospores are specialized resting structures that help *P. ramorum* survive adverse conditions. Laboratory experiments demonstrated that chlamydospores are very drought resistant but aren't very tolerant of high temperatures. At present, a fourth reproductive structure called an oospore (a sexual reproductive structure commonly associated with other *Phytophthora* species), has not been found in the United States.

*P. ramoru*m is a heterothallic organism, which means that two different mating types are required for sexual reproduction, genetic exchange, and production of oospores. Although sexual reproduction is not required for survival or multiplication of this pathogen, it adds a level of complexity to the organism. Because of the predominance of only one race (A2) in the United States, and another race (A1) in Europe, genetic exchange within this organism has only been observed under laboratory conditions. There is concern, however, that crossing of the two races A1 and A2 might create new, potentially more virulent hybrids since A1 and A2 differ in virulence and symptom expression. Thus, many questions remain unanswered regarding the biology, potential survival, or evolution of this newly encountered organism.

P. ramorum is considered to be a cool climate species with optimal growth at 68ºF. However, studies have demonstrated the ability of the organism to survive beyond these parameters, within the temperature range of 36 to 80ºF. Moisture is also important for survival, spread, and infection of *P. ramorum* as evidenced by natural infections of forests in coastal "fog belts" of California. Studies have shown that spores do not survive drying but survive for at least one month under moist conditions. Numerous studies on survival parameters of this organism are ongoing.

HOST RANGE

The plant host range for *P. ramorum* is broad and extremely diverse. The breadth of this host range is a characteristic that helps to distinguish *P. ramorum.* Most other *Phytophthora* species usually have more limited host ranges. The host list for *P. ramorum* includes herbaceous and woody species, and there are currently 31 "officially proven" or regulated hosts and 37 "officially associated" hosts. Proven or regulated hosts are hosts for which scientific tests have been completed to confirm they are host plants of *P. ramorum.* Associated hosts are species that have been found to be naturally infected with *P. ramorum* and for which testing has not been completed. Up-to-date lists are available at: http://www.aphis.usda.gov/ppq/ispm/pramorum/pdf_files/usdaprlist.pdf. Examples of proven hosts include viburnum, Japanese pieris, several species of oak, Douglas fir, and heather. The search for additional hosts is ongoing.

Greenhouse studies on seedlings of several important east coast tree species were conducted at the USDA ARS Foreign Disease-Weed Science Laboratory, Frederick, MD. These studies demonstrated the ability of *P. ramorum* to infect and produce cankers on many of the species tested. Included in this study were chestnut oak, white oak, and northern red oak, species commonly found in Connecticut forests. These findings serve to heighten our concern for East Coast forests.

SYMPTOMS

The disease symptoms associated with *P. ramorum* are very diverse and primarily determined by the host species. Symptoms can range from oozing, killing cankers on trunks and branches to foliar symptoms. Two distinct sets of symptoms associated with *P. ramorum,* and the diseases associated with these symptoms, have been called Sudden Oak Death and Ramorum Blight (Ramorum Dieback, Ramorum Leaf Blight). The disease symptoms characteristic of "Sudden Oak Death" result from lethal stem cankers in the bark, cambium, and outer xylem that expand and girdle the stem and kill the tree. These cankers often ooze and bleed. Tanoaks and certain oaks in the red oak subgenus exhibit these symptoms. Disease symptoms characteristic of "Ramorum Blight" are foliar blighting and shoot dieback. These are the typical symptoms exhibited by many non-oak host species. These symptoms are less severe than cankers and include leaf spots and blotches. In extreme cases, juvenile and mature plants with Ramorum Blight symptoms can be killed. Additionally, some species can exhibit both sets of symptoms. For example, *P. ramorum* can produce foliar symptoms as well as killing, bleeding cankers on tanoaks.

Unfortunately, disease symptoms characteristic of *P. ramorum* infections are often indistinguishable from other diseases or effects of insect problems that we frequently encounter in Connecticut woodlands and landscapes. Examples of some common look-alike problems are bacterial wetwood, boring insects, winter injury, mechanical injury, fungi, and other *Phytophthora* species, such as *P. cactorum*. Because there are many other tree and shrub diseases, injuries, and disorders that can cause similar symptoms, diagnosis of Sudden Oak Death or Ramorum Blight can be very confusing. If you are in doubt about what is causing a particular symptom on a plant, The Connecticut Agricultural Experiment Station (CAES) can provide assistance and expert advice.

DISEASE SPREAD

Because of the nature of this potentially devastating new disease, in 2002 the U. S. Forest Service generated a risk map for the United States. Each state was given a high, moderate, or low risk rating based on three key risk parameters. These parameters included: 1) presence of known host plants in overstory and understory of forests and woodlands; 2) climate; and 3) whether or not the state had nurseries that receive any proven or associated hosts of *P. ramorum* from the West Coast. Connecticut was determined to be at moderate risk. Additional risk maps are currently being constructed to include more comprehensive parameters.

P. ramorum has the potential to be dispersed over long distances through shipments of infected nursery stock. This pathogen also has the potential for spread via infested soils and potting media, and contaminated water. Additionally, timber, firewood, and woodchips stored under moist conditions can serve as long-distance vectors of the pathogen. Long distance spread resulting from shipment of infected nursery stock is precisely the situation Connecticut is facing due to the transport of infected nursery stock from the west coast.

Local spread of *P. ramorum* commonly occurs from movement of infected plant material, rain and irrigation water, and human activities (e.g., handling plants in nurseries, soil in shoes and tires of hikers and bikers, respectively).

One important and classic component of the epidemiology (study of the way diseases spread) of *P. ramorum* is the role played by different hosts. This component presents a "conundrum" since hosts exhibiting "Sudden Oak Death" type symptoms, which are often killed, usually aren't good sporulation hosts for *P. ramorum*. Whereas, hosts exhibiting "Ramorum Blight" type symptoms aren't usually killed, but are extremely good sporulation hosts for *P. ramorum*. These latter hosts are considered the epidemiologically important hosts in the spread of this disease since they are not killed by the infection and serve as a continuing source of the pathogen. Spores produced on the Ramorum Blight hosts are believed to infect and cause the killing cankers associated with SOD.

Although infections in natural and nursery situations have been subjected to federal regulations and quarantines and aggressive slash, burn, and spray policies, complete control and eradication has not been achieved. In part, these limitations can be attributed to our incomplete understanding of the life cycle and biology of this formidable pathogen.

WHAT IS HAPPENING IN CONNECTICUT? (Is Connecticut at risk?)

Scientists at CAES have been watching the situation in the West Coast since the first report of the mysterious, tree-killing disease in 2000. Additionally, the regulatory authority for Connecticut rests with CAES. There was an immediate response to the needs of Connecticut upon learning of shipments of potentially infected stock to the state. A team, informally called the "CT Ramorum Blight (SOD) Team," has been assembled that includes plant pathologists, state entomologists and plant inspectors from CAES as well as the state plant health director and inspectors from USDA Animal Plant Health Inspection Service- Plant Protection and Quarantine (APHIS-PPQ).

The potential spread of *P. ramorum* from the West Coast to other states was realized at a national level in 2004. The first "red flag" was raised in March 2004, when Connecticut was notified that a nursery had shipped potentially infected camellias from two California locations to approximately 39 states, including Connecticut. Nursery records indicated these shipments occurred as far back as 2003. Trace-forward investigations were immediately conducted. Trace-forward investigations or "trace-forwards" are based on the shipping records of the nursery that sent contaminated plants. They are used to track the path of the suspect plants to new locations, usually nurseries, and assess or test the condition of those plants in these locations. No camellia plants that had been part of the shipments to Connecticut tested positive for *P. ramorum*. The second "red flag" was raised in late October 2004, when Connecticut was notified about shipments of purportedly infected plants from Oregon. Some of these plants (shipped to many states from February- September 2004) were distributed to 56 outlets in Connecticut. The collective total of plants shipped to Connecticut was over 10,000 plants. Trace-forwards were immediately conducted on all of the plants that could still be located. Plants in three outlets tested positive for *P. ramorum.* Since many of the plants in the shipment had been sold as cash and carry, it was not possible to account for all of the infected plants. Thus, it is realistic to assume that some infected plants have been placed in the Connecticut landscape.

In addition to conducting trace-forward surveys, the CT Ramorum Blight Team participated in the National USDA APHIS-PPQ Ramorum Blight survey in 2003 and 2004 and the U.S. Forest Service Perimeter Survey in 2004. No plants in the surveys tested positive for *P. ramorum*. Participation in these surveys is also planned for 2005.

Detection and confirmation of *P. ramorum* is a complex task due to the use of three different testing processes. These procedures are Enzyme-Linked Immunosorbent Assay (ELISA), isolation and culture, and molecular detection using Polymerase Chain Reaction (PCR). ELISA is a serological test used as a prescreen to detect the presence or absence of a *Phytophthora* species in the plant. This test is not specific for *P. ramorum.* If the ELISA test is positive, then ELISA-positive plant tissues are placed on selective culture media. Once the organism is growing in culture, the identity of *P. ramorum* is confirmed by microscopic examination. Because of its unique morphological characteristics, *P. ramorum* is distinguished from other *Phytophthora* species, such as *P. cinnamomi or P. cactorum.* The latter two species are commonly found in Connecticut. Culture assays also verify the viability of the organism. Concurrent to culture, DNA extracted from the ELISA-positive samples is used for molecular detection using PCR. This technique confirms the presence of DNA originating from *P. ramorum* (and therefore identifies the organism) on the basis of a unique profile of DNA.

The detection of *P. ramorum* in plants shipped to Connecticut and likely planted in our landscape raises many concerns and questions, for many of which, we have no science-based answers. Will *P. ramorum* survive and grow in Connecticut? What hosts might contribute to survival and spread in the Connecticut environment? What is the importance of any soil phase in the ecology of the pathogen? How does it survive adverse conditions? Why are so many plant species susceptible to this pathogen? The list goes on. There are many informative web sites, fact sheets, publications, and a hotline on this topic. *Phytophthora ramorum*, the causal agent of Ramorum

Blight and SOD, is a serious pathogen, with significant potential for economic and ecosystem disruption. It is important to be concerned and aware of this pathogen since early detection is important for containment and management.

The "Ramorum Blight (SOD) Hotline" is 1-888-703-4457.
Among the helpful web sites are:
California Oak Mortality Task Force: http://www.suddenoakdeath.org/
NAPIS: http://ceris.purdue.edu/napis/pests/sod
USDA APHIS PPQ: http://www.aphis.usda.gov/ppq/ispm/pramorum/

Connecticut Agricultural Experiment Station Contact Information
Dr. Louis A. Magnarelli, Director, 203.974.8440
Dr. Kirby C. Stafford, State Entomologist, 203.974.8485
Dr. Victoria L. Smith, Deputy State Entomologist, 203.974.8474
Dr. Sharon M. Douglas, Plant Pathologist, 203.974.8499

March 2006 (revised)